

## Year 2003 Los Lunas Plant Materials Center Annual Technical Report

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# Effect of Gibberellic Acid and Standard Seed Treatments on Mountain Snowberry Germination<sup>1</sup>

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#### **Abstract**

Acid scarification, warm stratification, cold stratification, and soaks in gibberellic acid (GA<sub>3</sub>) were effective in promoting germination in mountain snowberry (Symphoricarpos oreophilus Gray [Caprifoliaceael]) from New Mexico, but treatment levels and interactions were important. The combination of a 30-min acid soak, 21-d warm stratification treatment, and 84-d cold stratification treatment (the shortest duration evaluated) was highly effective in promoting germination. Increasing cold stratification from 84 to 168 d increased germination, as did incubation in all concentrations (250 to 1000 ppm) of GA<sub>3</sub> but the benefit of longer cold stratification and GA<sub>3</sub> incubation was reduced for acid-scarified seeds. Acid scarification breaks physiological dormancy of the embryo and may allow maturation of the embryo during cold stratification to begin sooner. Timing of GA<sub>3</sub> application was also important. For seeds undergoing acid scarification followed by warm stratification followed by cold stratification, application of GA<sub>3</sub> prior to warm stratification resulted in less germination com- pared to application following warm stratification. In snowberry, early GA<sub>3</sub> application may result in GA<sub>3</sub> catabolism during warm stratification, reducing the concentration available during cold stratification.

#### **Key Words:**

Acid scarification, warm stratification, cold stratification, dormancy, GA<sub>3</sub>

A. NOMENCLATURE: ITIS (2001)

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#### Introduction

Mountain snowberry (*Symphoricarpos oreophilus* Gray [Caprifoliaceael) is an upright shrub occurring in numerous communities in montane regions of western North America (McMurray 1986). In New Mexico, mountain snowberry is found in ponderosa pine (*Pinus ponderosa* P. & C. Lawson [Pinaceae]) and mixed coniferous forests. This species occurs on open slopes and in the understory, occupies sites ranging from moist to dry, and grows on a wide range of soil pHs— characteristics that allow it to establish on disturbed sites (McMurray 1986). Mountain snowberry is a useful reclamation species, but propagation from seeds (technically, drupelets) is difficult.

A combination of mechanical, physiological, and morphological (embryo immaturity) mechanisms regulate snowberry seed dormancy. Initially, embryos are immature, requiring development that takes place during cold stratification (Flemion 1934). Restrictive seed coat fibers prevent embryo expansion and must be degraded before germination can occur (Flemion 1934, Pfeiffer 1934). In addition, maturation during cold stratification does not occur unless seed coats have previously been degraded by treatments such as acid scarification or prolonged (up to 70 d) warm stratification (Flemion 1934; Pfeiffer 1934). This requirement suggests that the seed coat exerts physiological control over the embryo, perhaps through growth-inhibiting substances. Acid scarification, warm stratification, and cold stratification have been used successfully to overcome dormancy in mountain snowberry and related species (Flemion 1934; Flemion and Parker 1942; Evans 1974; Rosner and others 2001). However, published literature regarding the use of gibberellic acid (GA) to promote germination of any snowberry species is lacking. Gibbereflic acid has been found to substitute for both warm stratification and cold stratification requirements in numerous species (Baskin and Baskin 1998). This study was carried out to examine the relative effectiveness of acid scarification, warm stratification, cold stratification, and GA<sub>3</sub> treatments on mountain snowberry germination. In addition the importance of timing of GA<sub>3</sub> application was tested

#### **Materials and Methods**

We conducted two experiments. The first experiment tested combinations of acid scarification, GA<sub>3</sub>, warm stratification, and cold stratification treatments applied in that order. The second experiment evaluated the timing of GA<sub>3</sub> incubation relative to the other treatments. For both experiments, seeds were collected from September through October 1997 at four locations (sources) in New Mexico (Table 1). Sources represented two locations at Molycorp Mine in Questa, New Mexico, and two locations in the Sangre de Cristo Mountains of northern New Mexico. Seeds were collected from 6+ plants at varying plant heights at each source. Fruits were soaked overnight in tap water, fermented for 48 h, mashed, and dried to facilitate cleaning. Seeds were dislodged from pulp in a rubbing box, separated from pulp in a South Dakota blower (Seedburo, Chicago, Illinois), and then stored at 5°C (41°F) in paper envelopes for 1 to 2 y prior to testing.

TABLE 1						
Mountain snowberry seed sources from New Mexico used in germination studies						
Seed source Latitude Location Elevation m (ft) Collection date						
Capulin	N 36° 42'	Questa	2987 (9800)	4 and 24 Sep		
Cabin	N 36° 42'	Questa	2408 (7900)	2 Sep		
Holman	N 36° 02'	Holman	2377 (7800)	7 Oct		
Rociada	N 35° 50'	Rociada	2377 (7800)	17 Oct		

TABLE 2					
Categorical analysis of variance table for mountain snowberry germination					
Source of variability	df	Chi-square	Observed significance		
Acid (A) <sup>a</sup>	1	299.6	< 0.001		
Warm (W) <sup>b</sup>	1	246.1	< 0.001		
Stratification (S) <sup>c</sup>	1	156.3	< 0.001		
$GA(G)^d$	3	71.0	< 0.001		
AXW	1	11.0	< 0.001		
AXS	1	51.5	< 0.001		
AXG	3	20.3	< 0.001		
WXS	1	12.5	< 0.001		
WXG	3	5.4	0.15		
SXG	3	10.9	1.01		
AXWXS	1	2.4	0.12		
AXWXG	3	3.6	0.31		
AXSXG	3	5.8	0.12		
WXSXG	3	2.1	0.56		
AXWXSXG	3	0.6	0.9		

<sup>&</sup>lt;sup>a</sup> Acid = acid scarification

incubation

#### **Experiment I**

We used a completely randomized design with a factorial treatment structure. Factors were acid scarification (0 or 30 min), GA<sub>3</sub> incubation concentration (0, 250, 500, or 1000 parts per million [ppm]), warm stratification (0 or 21 d), and cold stratification (84 or 168 d). Each treatment combination was tested with four 100-seed replications.

Concentrated sulfuric acid (Reagent ACS, 95% to 98%) was used to impose acid scarification treatments. Each 100-seed sample was added to 10 ml acid in a 50-mi beaker and stirred vigorously for 30 s. Seeds then soaked undisturbed for the treatment duration. Following treatment, seeds were rinsed under running tap water for I min. Seeds

<sup>&</sup>lt;sup>b</sup> Warm = warm stratification

<sup>&</sup>lt;sup>c</sup> Stratification = cold stratification

<sup>&</sup>lt;sup>d</sup> GA = gibberellic acid

underwent warm stratification mixed with moistened peat moss within self-sealing poly bags. Poly bags containing seeds and peat moss were stored in boxes in a laboratory, where air temperatures ranged from 21 to 24 °C (69 to 75 °F). Gibberellic acid treatments involved submersing the seeds in 20 ml of the appropriate GA<sub>3</sub> solution (GA<sub>3</sub> 90+%, Aldrich Chemical Company, Milwaukee, Wisconsin) for 24 h at room temperature. Seeds were cold stratified mixed with moistened peat moss in poly bags. Cold stratification temperatures fluctuated from an average daily low of -1 °C (29 °F) to an average daily high of 6 °C (41 °F).

Initially, seeds completing cold stratification were maintained within moistened peat moss in petri dishes for germination testing. Seedling emergence was to be the response variable. However, few seedlings emerged due to the fact that considerable germination occurred during cold stratification, and these germinants were weakened by pathogens and etiolation. The germination testing procedure was then changed, and samples tested prior to this change were dropped from the experiment. One replication of seeds undergoing 84 d of cold stratification in combination with all other treatments was

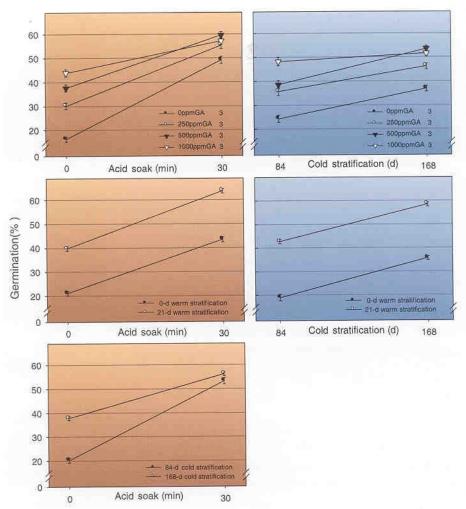


Figure 1: Significant (= 0.05) 2-factor interaction affecting mountain snowberry germination.

dropped, and two additional replications of seeds undergoing 84 d of cold stratification and 0 d of warm stratification in combination and with all other treatments were dropped as well.

For remaining seed samples, a revised germination testing procedure was used. Seeds were separated from peat moss, and germinated seeds were counted. Germinated seeds were defined as having their radicle protruding through the seed coat. Non-germinated seeds were rinsed under running tap water for 30 s and placed between two #1 grade qualitative filter papers (Whatman, Clifton, New Jersey) in a 150 mm (5.9 in) petri dish. Filter papers were moistened with distilled water. The petri dishes were placed in 15 X 16 cm (5.9 X 6.3 in) self-sealing poly bags and placed on FloraCart (Grower's Supply Company, Dexter, Michigan) plant stands located in the laboratory. Two 40-watt Sylvania Grow Lux fluorescent bulbs were suspended 30 cm (11.8 in) over the petri dishes.

The light cycle was a 10-h light period followed by a 14-h dark period. Air temperature immediately above the petri dishes ranged from an average daily low of 21 °C (69 °F) to an average daily high of 30 °C (85 °F). Germination was monitored after 7, 14, 21, and 28 days.

#### **Experiment 2**

The second experiment utilized a completely randomized design with a factorial arrangement of seed source (4) and GA<sub>3</sub> timing treatments (4) described below. Each treatment/source combination was replicated with four 100-seed samples. All seeds underwent acid scarification followed by warm stratification followed by cold stratification. Interposed among these treatments, seeds underwent incubation in GA<sub>3</sub>. Acid scarification (30 min), warm stratification (21 d), and cold stratification (168 d) treatment levels were standard for all seeds.

	TABLE 3					
Main effects of acid scari	Main effects of acid scarification, warm stratification, cold stratification, and gibberellic acid incubation on mountain snowberry					
Treatment level Germination Standard error Improveme to contr						
Acid scarification (min)						
0	32.0 b	0.7				
30	55.3 a	0.7	73			
Warm stratification (d)						
0	32.3 b	0.7				
21	51.7 a	0.7	60			
Cold stratification (d)						
84	38.6 b	0.8				
168	47.0 a	0.6	28			
Gibberellic acid (ppm)						
0	32.7 c	1				

TABLE 3						
Main effects of acid scar			ution, and gibberellic acid			
	incubation on mo	ountain snowberry				
Treatment level Germination Standard error Improvement relative to control (%)						
250	42.7 b	1	31			
500	48.5 a	1	48			
1000	50 5 a	1	54			

<sup>&</sup>lt;sup>a</sup> Germination percentage within treatments followed by the same letter are not significantly different at

Acid scarification was imposed by the methods described above. Warm stratification treatment involved placing seed samples into 2 X 10 cm (0.8 X 3.9 in) synthetic screen pouches, which were inserted into moist peat moss within self-sealing poly bags. Seeds were placed in a single layer in the pouch, and the pouch was placed into the peat moss ensuring adequate seed-peat moss contact. Samples were maintained at room temperature, which fluctuated between mean daily lows of 22 +/- 0.1 °C (71 +/- 0. I °F) and mean daily highs of 23 +/-0.1 °C (74 0.1°F), for 21 d. Finally, seeds (still within screen pouches in peat moss) underwent 168 d of cold stratification in a walk-in cooler.

Cooler temperatures fluctuated from an average daily low of -1 +/- 0.1 °C (30 +/- 0.1 °F) to an average daily high of 5 +/- 0.1 °C (42 +/- 0.1 °F). Gibberellic acid treatments involved submerging seeds in 20 ml of 500 parts per million (ppm  $GA_3$  solution) for 24 h at room temperature.

	TABLE 4						
	Mean germination percentages and standard errors for mountain snowberry seeds undergoing combinations of acid scarification, gibberellic acid ( $GA_3$ ) incubation, warm stratification, and cold stratification treatments						
Acid Soak (min)	Warm Stratification (d)	Cold stratification (d)	Gibberellic acid (ppm)	Sample size (number of seeds)	Mean germination (%)	Standard error	
0	0	84	0	100	2.0	1.4	
0	0	84	250	100	6.0	2.4	
0	0	84	500	100	3.0	1.7	
0	0	84	1000	100	8.0	2.7	
0	0	168	0	400	14.5	1.8	
0	0	168	250	400	25.3	2.2	
0	0	168	500	400	29.0	2.3	
0	0	168	1000	400	32.0	2.3	
0	21	84	0	300	10.7	1.8	
0	21	84	250	300	22.3	2.4	
0	21	84	500	300	24.7	2.5	
0	21	84	1000	300	44.0	2.9	

a = 0.05 for acid scarification, warm stratification, and cold stratification, or a = 0.083 (a = 0.05/6) for gibberellic acid treatments.

T	A	R	T	Æ	4

Mean germination percentages and standard errors for mountain snowberry seeds undergoing combinations of acid scarification, gibberellic acid  $(GA_3)$  incubation, warm stratification, and cold stratification treatments

Acid Soak (min)	Warm Stratification (d)	Cold stratification (d)	Gibberellic acid	Sample size (number of seeds)	Mean germination (%)	Standard error
			(ppm)			2.2
0	21	168	0	400	25.8	2.2
0	21	168	250	400	47.0	2.5
0	21	168	500	400	64.5	2.4
0	21	168	1000	400	64.8	2.4
30	0	84	0	100	17.0	3.8
30	0	84	250	100	36.0	4.8
30	0	84	500	100	42.0	4.9
30	0	84	1000	100	37.0	4.8
30	0	168	0	400	45.5	2.5
30	0	168	250	400	45.8	2.5
30	0	168	500	400	49.3	2.5
30	0	168	1000	400	43.8	2.5
30	21	84	0	300	48.0	2.9
30	21	84	250	300	59.0	2.8
30	21	84	500	300	63.3	2.8
30	21	84	1000	300	69.7	2.7
30	21	168	0	400	61.8	2.4
30	21	168	250	400	66.8	2.4
30	21	168	500	400	71.3	2.3
30	21	168	1000	400	66.0	2.4

The four GA<sub>3</sub> timing treatments were:

- 1. Acid scarification/ GA<sub>3</sub> incubation/ warm stratification/ cold stratification;
- 2. Acid scarification/ warm stratification/ GA<sub>3</sub> incubation/ cold stratification;
- 3. Acid scarification/ warm stratification/ 28-d cold stratification/ GA<sub>3</sub> incubation/ 140-d cold stratification;
- 4. Acid scarification/ warm stratification/ 56-d cold stratification/ 24-h GA<sub>3</sub> incubation/ 112-d cold stratification.

Following completion of treatments, germinated seeds were counted and non-germinated seeds were incubated to test for further germination. The filter paper method of germination testing described above was used and the conditions were the same.

TABLE 5				
Categorical analysis of variance table for mountain snowberry germination response to timing of				
gibberellic acid incubation, seed source, and the interaction of both factors				
Component	df	Chi-square	Observed significance	
Seed source (5)	3	850.9	< 0.001	
Treatment timing (1)	3	21.4	< 0.001	
SXT	9	28.3	< 0.001	

#### **Statistical Analysis**

For both experiments, categorical analysis (SAS PROC CAT-MOD; SAS Institute 1989) was used to determine treatment differences in germination using factorial treatment structures. In addition, data was analyzed separately by seed source for the second experiment. Categorical analysis is a generalization of the chi-square ( $x^2$ ) test of homogeneity, which uses the "logit"—the natural log of the ratio of germinated to nongerminated seed for each treatment—as the response. There is no need to transform germination data using this analysis. Due to some low cell counts, the generalized least square approach was used to calculate  $x^2$  test statistics for the first experiment, whereas maximum-likelihood analysis was used in the second experiment. Observed significance levels less than a=0.05 were considered significant. Percentages and standard errors were calculated for main effects and interaction combinations. Approximate pairwise Z statistics were used to conduct comparisons of main effects having more than two treatment levels. A conservative alpha value of 0.05 divided by the number of comparisons was chosen (equivalent to Bonferroni multiple comparison method).

### Results

#### **Experiment I**

Acid scarification, GA<sub>3</sub> incubation, warm stratification, and cold stratification affected germination (Table 2).

For each main treatment effect, as treatment intensity increased, germination increased (Table 3). All first-order interactions among treatments (with the exception of the interaction between warm stratification and GA<sub>3</sub> incubation) impacted germination (Table 2). Certain 2-factor interactions such as warm stratification X cold stratification and warm stratification X acid scarification were statistically significant due to the large sample size resulting from averaging across the other two factors, but these differences were not practically significant. In all cases, the combination of two treatments (averaged over all levels of the other two factors) improved germination relative to each treatment individually (Figure 1). However, certain treatment combinations were only marginally more effective than the better treatment alone. For example, increasing cold stratification length from 84 to 168 d nearly doubled the germination of non-scarified seeds but had little effect on germination of seeds that had undergone a 30-min acid soak.

Likewise, gibbeirellic acid improved the germination of non-scarified seeds nearly threefold, but improvement was considerably less when this treatment was applied to

acid-scarified seeds. Mean germination percentages and standard errors for all treatment combinations are presented in Table 4.

#### **Experiment 2**

Timing of gibberellic acid application affected germination response, but seed source had a much larger impact (Tables 5 and 6). The influence of timing of gibberellic acid treatment differed slightly among seed sources (Table 6). For all but the Capulin seed source, application of GA<sub>3</sub> prior to warm stratification treatment resulted in less germination compared to application at some point following warm stratification treatment. Seeds from the Capulin source germinated equally well regardless of timing of application.

	TABLE 6		
Effect of timing of inci		lion gibberellic acid (GA3) o	n mountain
	snowberry germination l	•	
Seed source	GA treatment <sup>a</sup>	Mean germination <sup>b</sup>	Standard
	timing	(%)	error
Capulin	1	50.0 a	2.5
	2	51.5 a	2.5
	3	43.8 a	2.5
	4	43.8 a	2.5
	pooled	48.5	1.2
Cabin	1	21.5 b	2.1
	2	30.0 a	2.3
	3	32.3 a	2.3
	4	24.8 ab	2.2
	pooled	27.1	1.1
Holman	1	62.8 b	2.3
	2	71.3 ab	2.2
	3	75.0 a	2.2
	4	73.5 a	2.0
	pooled	70.6	1.1
Rociada	1	68.8 b	2.3
	2	74.5 ab	2.2
	3	74.5 ab	2.2
	4	78.8 a	2.0
	pooled	74.1	1.1

<sup>&</sup>lt;sup>a</sup> 1 = GA applied following acid scarification.

<sup>2 =</sup> GA applied following warm stratification.

<sup>3 =</sup> GA applied following 28 d cold stratification.

<sup>4 =</sup> GA applied following 56 d cold stratification.

Mean germination percentages within a source followed by the same letter are not significantly different at a = 0.05/6

#### **Discussion**

Consistent with previous work on common snowberry (*Symphoricarpos albus* var. *albus* [L.] Blake, formerly *Symphoricarpos racemosus* Michx.) (Flemion 1934; Evans 1974), Indian currant snowberry (*Symphricarpos orbiculatus* Moench) (Flemion and Parker 1942; Evans 1974), and mountain snowberry (Rosner and others 2001), acid scarification and warm stratification treatments were effective in promoting germination, and a combination of treatments was most effective. Both treatments degrade restrictive seed coat fibers and break physiological control over the embryo exerted by the seed coat (Flemion 1934; Pfeiffer 1934). Combined treatment is thought to allow more thorough seed coat degradation without the occurrence of embryo damage (Pfeiffer 1934).

Published literature on North American snowberry species recommends 120 to 180 d of cold stratification (Flemion 1934; Flemion and Parker 1942; Evans 1974). We found that increasing cold stratification duration from 84 to 168 d improved germination on average, but few acid scarified seeds required cold stratification beyond 84 d. Acid scarification may allow embryo maturation to begin earlier in the cold stratification process, by breaking seed coat-imposed physiological control of the embryo.

Warm stratification, on the other hand, improved germination about as well in combination with 84- and 168-d cold stratification treatments. Published literature recommends 90- to 120-d treatment durations when warm stratification is used instead of acid scarification (Flemion 1934; Evans 1974), but shorter durations when used in conjunction with acid scarification (Flemion 1934; Flemion and Parker 1942). Short durations of warm stratification, such as 21 d used in this study, may be inadequate to thoroughly degrade restrictive seed coat fibers initially, but fungi infecting the seed coat during incubation at room temperature may continue to degrade seed coat fibers throughout the cold stratification process. In addition, short periods of warm stratification may stimulate some germination-promoting metabolic processes such as reduction in levels of abscisic acid (ABA) and alterations in cellular development (Chien and others 1998).

The role of gibberellic acids in promoting germination is highly variable among taxa (Li and Ross 1990; Karssen 1995; Chien and others 1998). Among some species with cold stratification requirements, ABA and GAs are thought to play antagonistic roles in the maintenance and breaking of dormancy (Nicolas and others 1996).

Exogenous GA<sub>3</sub> application has been shown to substitute for cold stratification in some species (Powell 1987; Nicolas and others 1996; Baskin and Baskin 1998; Chien and others 1998). GA<sub>3</sub> substituted for at least a portion of the cold stratification requirement in mountain snowberry in our study. Germination of GA<sub>3</sub> treated seeds undergoing 84 d of cold stratification was as high or higher (depending on treatment level) than germination of untreated seeds cold stratified for 168 d. Cold stratification is believed to activate the gibberellin-synthesizing mechanism (Powell 1987), but in some species cold stratification may also increase seed sensitivity to GAs (Chien and others 1998). In this study, erogenous GA<sub>3</sub> may have substituted for endogenous GAs typically produced later in the cold stratification period, or the concentration of applied GA<sub>3</sub> may have been high

enough to overcome insensitivity to GAs. Gibberellic acid application also improved germination of seeds cold stratified 168 d, suggesting that some seeds had a cold stratification requirement greater than 168 d, or that GA improved germination by some means other than as a substitute for cold stratification. Exogenous GA<sub>3</sub> application was less effective when used in conjunction with acid scarification. By reducing the necessary duration of cold stratification, acid scarification would be expected to reduce the efficacy of treatments substituting for cold stratification.

A risk with GA treatments is that some developmental processes taking place during cold stratification may be bypassed. During cold stratification, most aspects of metabolism are affected in some way (Mayer and Poijakoff-Mayber 1982).

Also, high post-germination levels of GAs can interfere with proper seeching growth. GA<sub>3</sub> was used as a substitute for cold stratification in Judas tree (*Cercis siliquastrum* L. [Fabaceael) and resulting seedlings had reduced root-to-shoot ratios and problems maintaining a favorable water balance (Rascio and others 1998). In that species, reserve mobilization normally begins after germination, but erogenous GA<sub>3</sub> treatment resulted in reserve mobilization prior to germination. Further research is needed to determine if all or only part of snowberry's cold stratification requirement can be bypassed by GA<sub>3</sub> treatment and to assess the effect of such treatment on the growth and development of the resulting seedling.

We found that GA<sub>3</sub> was most effective when applied at any time after completion of warm stratification for 3 of 4 seed sources. Gibberellic acid has been shown to break physiological dormancy related to warm stratification requirements in some species (Baskin and Baskin 1991; Baskin and Baskin 1998). In snowberry, however, the warm stratification requirement may be related to multiple dormancy mechanisms. Warm stratification facilitates degradation of the seed coat (Pfeiffer 1934) but may improve germination through other mechanisms-warm stratification improved germination of mountain snowberry seeds that had undergone supra-optimal acid scarification treatment (Rosner and others 2001). If the benefit of GA<sub>3</sub> treatment was related more to processes taking place during cold stratification than warm stratification, it would be expected that GA<sub>3</sub> application prior to warm stratification would be less effective than application made after warm stratification; GA<sub>3</sub> catabolism during warm stratification treatment would reduce the concentration available during cold stratification treatment.

## **Practical Applications**

Gibberellic acid treatment of mountain snowberry seeds can be used to avoid either sulfuric acid scarification or lengthy (> 84 d) cold stratification treatments but not both. To bypass acid scarification, seeds should be warm-stratified for 21 d, incubated in 500 or 1000 ppm GA $_3$  for 24 h, and stratified 168 d. When seeds are scarified for 30 min in sulfuric acid, warm stratified for 21 d, and then incubated in 250 to 1000 ppm GA $_3$  for 24 h, the cold stratification duration can be reduced to 84 days with little reduction in germination.

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## Refinement and Stratification of Thinleaf Alder and Water Birch Seeds from New Mexico<sup>6</sup>

By: Cindy L. Jones<sup>7</sup>, John Harrington<sup>8</sup>, and David Dreesen<sup>9</sup>

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#### Abstract

For multiple seed collections of thinleaf alder (*Alnus tenuifolia* (Nutt.) Breitung [Betulacael]) and water birch (*Betula occidentilis* (Hook.) [Betulacael]), response to IDS (Incubation, Drying, and Separation), gravity separation, and stratification was highly variable among seed collections. In thinleaf alder, drying periods of 18 or 24 h following a 24-h incubation period were comparable to dry seed separation in petroleum ether for increasing percentage of filled seeds. In water birch, IDS treatments resulted in lower percentages of filled seeds than separation in 95% ethanol. Overall, cold (5 °C [41°F]) wet stratification for 56 d improved water birch germination from 11% to 16%. In thinleaf alder, response to a 56-d stratification ranged from 0% to 16% germination improvement. Using separated seed in combination with appropriate stratification length achieved the largest improvements in germination. Treatment selection is discussed in relation to optimizing use of limited greenhouse space and seed supply.

#### **Key Words:**

IDS separation, gravity separation, stratification

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### Introduction

Thinleaf alder (*Alnus tenuifolia* (Nutt.) Breitung [Betulacael]) occurs in mountain ranges of western North America, typically growing as a shrub or small tree in riparian areas at elevations of 1520 to 3040 m (5000 to 10,000 ft) (Vines 1960). Thinleaf alder fixes atmospheric nitrogen via a symbiotic relationship with the actinomycete *Frankia* spp. (Virtanen 1957; Bond 1976) and the species clump-forming habit is valuable in erosion control and disturbed land revegetation (Vines 1960).

Water birch (*Betula occidentalis* (Hook.) [Betulacae]) occurs naturally from southern California and New Mexico north to Alaska, Manitoba, and North Dakota, but is absent along the Pacific Coast mountain ranges and portions of the Sierra Nevada Mountains (Uchytil 1989). It occurs as a shrub or small tree along streams or in moist canyons, and occasionally on dryer sites of the mountain West at elevations of 1500 to 2700 m (4900 to 8900 ft) (Vines 1960).

Interest in these species for revegetation applications has recently increased because both species grow fast, produce prolific amounts of seeds, and have short life cycles (Elias 1980). While propagation of alder and birch species has been studied, literature pertaining to propagation requirements of thinleaf alder and water birch is lacking. Seeds of Betulaceae are characteristically very small and light (1500 to 2500 seeds/g [42500 to 70800 seeds/oz]) and may have a winged integument for wind dispersal (Vines 1960). Seed quality and germination capacity are often very low, as it is difficult to separate sound from empty seeds using standard size and density separation techniques on small, winged seeds (Brinkman 1974; Schopmeyer 1974).

The IDS method (Incubation, Drying, and Separation) for separating viable filled seeds from unfilled or nonviable seeds has been successful for both coniferous and hardwood species (Simak 1983; Sweeney and others 1991; Downie and Wang 1992; Falleri and Pacella 1997). In the IDS method, after imbibition, empty or nonviable filled seeds lose water more rapidly than viable filled seeds during drying. The differential moisture content during drying allows separation by flotation or other density separation methods. Alnus and Betula seeds exhibit various degrees of dormancy that can be broken by cold stratification and/or germination under red light (Brinkman 1974; Schopmeyer 1974; Dirr and Heuser 1987; Young and Young 1992). Pretreatment requirements for germination of alder seeds are variable between and within species. Stratification periods of 60 to 180 d are recommended for many alder species (Dirr and Heuser 1987). However, stratification treatment of thinleaf alder did not improve germination percentage (Young and Young 1992). The purpose of our study was to determine the effectiveness of IDS and gravity separation techniques to increase the percentage of filled seeds in thinleaf alder and water birch. Secondly, we examined using separation techniques in combination with varying levels of stratification on germination of thinleaf alder and water birch. To achieve these objectives, 2 experiments using multiple seed collections of both thinleaf alder and water birch from the southern Rocky Mountains were conducted. First, a refinement experiment tested the effects of multiple separation treatments on percentage of filled seeds generated in sinking and floating fractions and the ability of these treatments to recover filled seeds

in the original samples. Secondly, a germination experiment tested the efficacy of separation and stratification treatments on germination.

#### **Materials And Methods**

#### Seed Collections

Thinleaf alder strobiles were collected in October and November of 1998 in Catron County, New Mexico, near Luna (Cottonwood Canyon Campground) and Reserve (Head of the Ditch Campground) and in Taos County, New Mexico (Red River Canyon near the Molycorp molybdenum mine) (Table 1). Seed lots from Red River Canyon (RRC, Moly) included collections from distinct stands within a 4-km (2.5-mile) stretch of the canyon. Bracts were collected when <10% of the strobiles were beginning to open. The Luna, Reserve, and both Red River seed collections (RRC-1, RRC-2) of thinleaf alder were used in the refinement study. The Luna, Reserve, and RRC (RRC-1 and RRC-2 pooled) collections and a commercial seed collection, collected in fall 1998 in Chaffee County, Colorado, were used in the germination study. Strobiles were kept cool and allowed to dry for several weeks. Thinleaf alder seeds were separated from opened strobiles by rubbing on a coarse screen.

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Seed collection	Seed collection locations and baseline percentage of filled seed for thinleaf alder and water birch seed used in experiments.					
Species	Collection name	Baseline percentage of filled seed	Location description	Elevation (meters) <sup>a</sup>	Latitude longitude <sup>b</sup>	
Thinleaf Alder	Luna	23	Head of the Ditch Campground	2134	N33°49' W108°59'	
	Reserve	27	Cottonwood Canyon	1829	N33°37' W108°55'	
	RRC-1	1	Red River Canyon (river bottom)	2377	N36°41.08' W105°29.85'	
	RRC-2	1	Red River Canyon (up slope)	2492	N36°41.67' W105°29.85'	
	Chaffee	54	West of Poncha Springs, Colorado	na	N38°31' W106°05'	
Water Birch	RRC-3	7	Red River Canyon	2377	N36°41.081' W105°31.52'	
	Moly-1	4	Molycorp-tailings road	2361	N36°41.23' W105°32.36'	
	Moly-2	5	Molycorp-lower overburden pile	2492	N36°41.67' W105°29.85'	
	Moly-3	6	Molycorp-Safety Berm	2403	N36°40.86' W105°32.36'	
	Chaffee	30	West of Poncha Springs, Colorado	na	N38°31' W106°05'	
<sup>a</sup> Conversion:	1m = 3.3  ft					

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Seed collection locations and baseline percentage of filled seed for thinleaf alder and water birch seed used in experiments.

		B	ase	lin

Col	llection percentag	ge Location	Lievation	Latitude
Species nar	ne of filled se	eed description	(meters) <sup>a</sup>	longitude <sup>b</sup>

b Latitude and longitude values for the Lunas, Reserve, and Chaffee collections were determined from a topographical map and recorded to the nearest whole minute. Values for the RRC and moly collections were obtained with a global positioning receiver and recorded to the nearest 100<sup>th</sup> of a minute.

Birch strobiles were collected in October and November of 1998 in Taos County, at 4 locations (RRC-3, Moly-1, Moly-2, and Moly-3) in the Red River Canyon near the Molycorp molybdenum mine (Table 1). Bracts were collected when <10% of the strobiles were beginning to open. All 4 collections were used in the refinement study. The RRC (RRC-3 and Moly-3 pooled), Moly-1, and Moly-2 collections of water birch along with commercial seeds collected in fall 1998 in Chaffee County, Colorado were used in

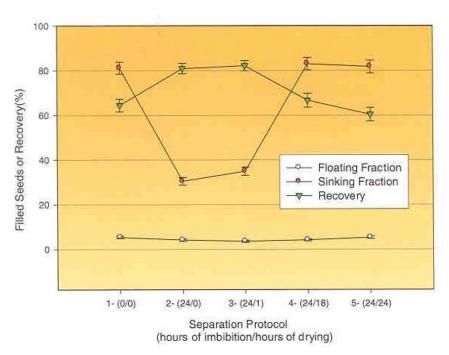


Figure 1: Effect of separation protocol on percentage of filled seeds in sinking and floating fractions, and percentage recovery of filled seeds present in the original sample by the sinking fraction for thinleaf alder. Error bars represent  $\pm$  one standard error. Error bars are too small to be visible in some cases.

the germination study. Water birch strobiles were kept cool and allowed to dry for several weeks, allowing seeds to release from bracts.

Prior to any treatments, all seed collections were examined for percentage of filled seeds (baseline percentage of filled seeds) using a dissecting microscope at 30X magnification (Berry and Torrey 1985). Baseline percentage of filled seeds for thinleaf alder was estimated by averaging the results of 25 samples of 100 seeds for each seed collection (Table 1). Baseline percentage of filled seeds for water birch was determined by averaging the results of 15 samples of 50 seeds for each seed collection (Table I). For both species, working samples were drawn from seed collections by mixing the entire collection thoroughly, and halving and rehalving the collection until the necessary quantity of seeds was obtained. Fifty or 100-seed samples were counted out of the working sample following a thorough remixing, and these samples were randomly assigned to treatments.

#### **Separation Media**

Initial trials with ethanol (specific gravity = 0.79) and water were unsuccessful at separating filled and unfilled thinleaf alder seeds, either using IDS or when separating city seeds. In both cases, filled and unfilled seeds remained in the floating fraction. Initial trials showed that petroleum ether (specific gravity = 0.60) was more effective as a separation medium.

Gravity separation in water was ineffective for separating water birch seeds. However, both ethanol and petroleum ether effectively separated dry water birch seeds. Petroleum ether, ethanol, and water were somewhat effective in separating water birch seeds previously treated by the IDS method. Ethanol was chosen as the separation medium because of cost, effectiveness, and availability.

#### **Seed Refinement Study**

Separation treatments for thinleaf alder seeds included density separation of dry seeds in petroleum ether (control) and IDS separation of 24-h imbibed seeds in petroleum ether following drying periods of 0, 1, 18, or 24 h. Five replications of 100 seeds were performed for each treatment.

Separation treatments for water birch seeds were density separation of dry seeds in 95% ethanol (control), and IDS separation of 12-h imbibed seeds in 95% ethanol following drying periods of 0, 0.5, 1, and 2 h. Three replications of 50 seeds were preformed for each treatment.

All seeds were imbibed by submersion in a 384 (10-gal) glass aquarium filled with distilled water and equipped with an aeration pump and filter. Seeds were packaged in filter paper and enclosed in weighted wire cages to keep them submerged. Following imbibition, seeds were thoroughly blotted and placed on dean clean filter paper. The drying incubation was performed in a closed chamber consisting of a 38-1 (10-gal) aquarium with polyethylene film taped over the top. Constant humidity inside the chamber was obtained using CaC1<sub>2</sub>·6H<sub>2</sub>O salt in a saturated solution prepared by adding 5000 g CaC1<sub>2</sub>·6H<sub>2</sub>O to 3.0 1 of distilled water (Young 1967; Slavik 1974). Seeds were

placed on filter paper and suspended on a screen above the solution. Humidity remained at 50% and was monitored using a hygrometer.

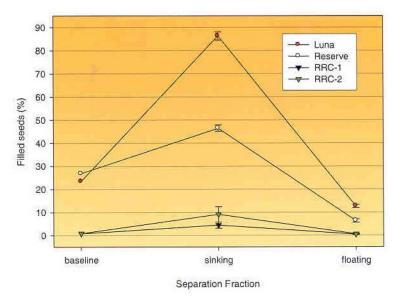


Figure 2: Effect of separation treatment on thinleaf alder percentage of filled seeds by seed collection. Baseline percentage of filled seeds is included for reference.

After IDS drying, seeds were placed briefly in petroleum ether or ethanol and the solution was vigorously stirred for 20 s to separate seeds. Floating seeds were removed from the surface, rinsed, and placed on clean moistened filter paper within plastic bags. Sinking seeds were strained through a net and packaged a similar manner. Percentage of filled seeds in each fraction was determined by dissection. Percentage of the total filled seeds in the original sample recovered in the sinking fraction (percent recovery) was calculated using Equation 1.

#### **Equation 1**

#### **Germination Study**

This study tested the factorial combination of separation, collection, and stratification treatments for both species. The 3 seed separation treatments used for thinleaf alder were the:

- 1. Floating seed fraction following 24-h imbibition and 18-h drying using petroleum ether
- 2. Sinking fraction using the separation above
- 3. Seed imbibed for 24-h with no separation.

Seed separation treatments used for water birch included the:

- 1. Floating seed fraction following 12-h imbibition using 95% ethanol
- 2. Sinking fraction following 12-h imbibition using 95% ethanol
- 3. Seed imbibed for 12-h with no separation.

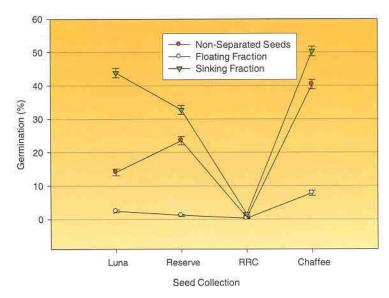


Figure 3: Effect of separation treatment on thinleaf alder germination by seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

For thinleaf alder, we used stratification lengths of 0, 28, and 56 d, while for water birch stratification lengths were 0, 2 1, and 56 d. For stratification, seeds were placed between layers of paper towel, moistened with 25 ml (0.3 fl oz) of distilled water, and sealed in polyethylene bags. Bags were stored in a cooler with an average temperature of 5 °C (41 °F) (temperature ranged from 4.0 to 6.0 °C [38 to 43 °F]) for the respective treatment durations. Initiation of stratification treatments was staggered so that 0 treatments came out of stratification concurrently.

The factorial arrangement of collection, seed separation, and stratification treatments resulted in 36 treatment combinations for each species. Each treatment combination was replicated with four, 100-seed samples. Following stratification, seeds were sown in Ray Leach Super Cells (Steuwe & Sons Inc; Corvallis, Oregon) containing a 2:1:1 ratio by volume of peat:perlite:vermiculite with Osmocote 14N:14P<sub>2</sub>O<sub>2</sub>: 14K<sub>2</sub>O slow release fertilizer at a rate of 4 kg/ml<sup>3</sup> (6.75 lb/yd<sup>3</sup>). Five seeds were sown per container with 80 containers per treatment combination. Containers were placed in the center of a 10 in (33 ft) by 13 in (43 ft) greenhouse for germination. Treatments were arranged in a randomized complete block design with 4 blocks per species. Each block included one, 20-container (100-seed) replication of each treatment combination. Germination conditions were ambient light (average 13.5 h/d and 70% relative humidity, with an average daytime temperature of 24 °C (75 °F) (daytime temperature range 20 to 27 °C [68 to 80 F]), and an average night temperature of 22 °C (70 °F) (nighttime temperature

range 20 to 23 °C [69 to 75 °F]). Cells were watered at 2-h intervals 6 times daily. Germination was recorded 7, 14, 21, and 28 days after planting.

#### **Data Analysis**

Categorical analysis of variance using SAS PROC CATMOD (SAS Institute 1989) was performed on all data. Categorical analysis of variance fits linear models to functions of response frequencies, and partitions the variation among those functions into various sources (SAS Institute 1989). This procedure is appropriate for binomial type of probability distribution (seeds filled or not filled) and does not require data transformation. For the refinement study, the response variables were percentage of filled seeds in the sinking and floating fractions, and the percentage of filled seeds recovered from those present in the baseline sample. Data were analyzed as a 5 (preparation protocol) by 2 (separation fraction) by 4 (seed collection) factorial for each species. For the germination study, the response variable was germination percentage, and data were analyzed as a 3 (separation treatment) by 3 (stratification duration) by 4 (seed collection) factorial for each species.

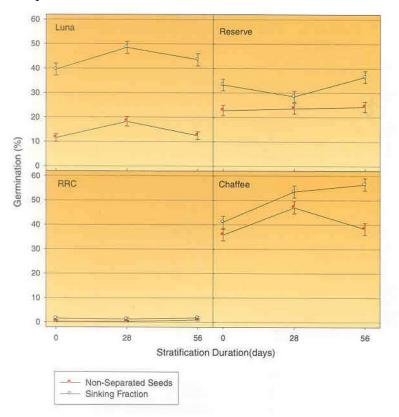


Figure 4: Thinleaf alder germination percentage as influenced by stratification length, separation treatment, and seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

For both experiments, marginal percentages (main effect and interaction combinations) along with standard errors were calculated using PROC MEANS SAS Institute 1989). Pairwise Z-tests (a = 0.05) were used to separate mean percentages. This method of percentage separation is analogous to Fisher's LSD.

#### Results

#### **Thinleaf Alder Refinement Study**

Preparation protocol, seed collection, separation fraction, and interactions between fraction and collection and fraction and protocol influenced the percentage of filled seeds. The percentage of filled seeds was low in the floating fraction (mean = 4%) and varied in the sinking fraction across preparation protocols from 30% to 83% (Figure 1). The control and the two IDS treatments with the longest drying durations (I 8 and 24 h) had higher percentages of filled seeds in the sinking fraction than IDS treatments without drying or a 1-h drying period. Overall, floating fractions had a lower percentage of filled seeds (4%) than sinking fractions (47%).

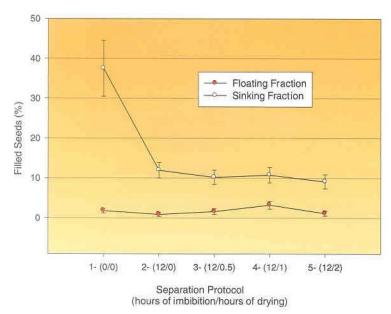


Figure 5: Water birch percentage of filled seeds as influenced by separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

Seed collections diffused in baseline percentage of filled seeds, percentage of filled seeds in the sinking fraction, and percentage of filled seeds in the floating fraction (Figure 2). Separation improved the percentage of filled seeds in the sinking fraction compared to the baseline by almost 4X for the Luna collection, 2X for the Reserve collection, 6X for the RRC-1 collection, and 1 OX for the RRC-2 collection. In contrast to the percentage of filled seeds in the sinking fraction, percent recovery was increased with IDS treatments having either no drying or 1-h drying (Figure 1). Percent recovery also varied among collections, 32% for RRC-2, 52% for RRC-1, 54% for Luna, and 88% for Reserve.

#### **Germination Study**

Seed collection, separation treatment, the interaction of both these factors, and the 3-way interaction of stratification length, separation treatment, and seed collection affected germination percentage. Averaged over all treatments, the RRC seed collection germinated poorly (1%) compared to other seed collections, while the Chaffee seed collection had the highest germination (33%). The Reserve and Luna seed collections

germinated at 19% and 20%, respectively. This result follows baseline percentages of filled seeds for these collections (Table 1). Seeds in the sinking fraction had the highest germination at 32%, compared to 3% for the floating fraction and 19% for non-separated seed. The effect of separation treatments varied among seed collections (Figure 3). Seed collections with higher overall germination percentages (and baseline filled percentages)-Luna, Reserve, and Chaffee also had the largest improvements in germination, 30%, 9%, and 10%, respectively. Germination of the RRC-1 seed collection was poor regardless of separation treatment, however, this collection did have a significant 1% improvement in germination in the sinking fraction. Some germination occurred in the floating fraction, with the Chaffee collection having the greatest germination in this fraction (8%). The influence of stratification on germination was both seed collection- and separation fraction-dependent. In the Chaffee collection the germination response to stratification differed for the sinking and non-separated fractions. For seeds in the sinking fraction, both 28- and 56-d stratification periods improved germination, whereas only 28-d of stratification improved germination in non-separated seeds (Figure 4). This later response is similar to the trend observed in both non-separated and sinking fractions of seeds in the Luna collection. Stratification did not significantly affect germination in either fraction of the Reserve or RRC collections of thinleaf alder.

#### **Water Birch**

#### **Refinement Study**

Preparation protocol, separation fraction, and the 2-factor interactions between separation fraction and seed collection and separation fraction and protocol influenced the percentage of filled seeds. As was the case with thinleaf alder, percentage of filled water birch seeds was low in the floating fraction, but varied with the preparation protocol in the sinking fraction (Figure 5).

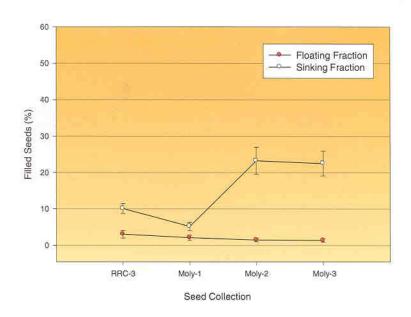
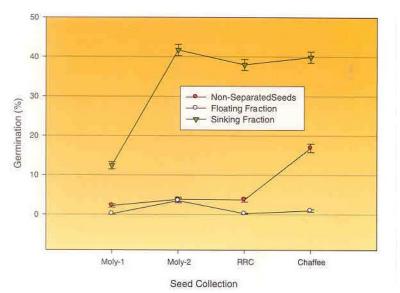


Figure 6: Water birch percentage fill as influenced by seed collection and separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

All 4 IDS treatments reduced the percentage of filled seeds in the sinking fraction to less than one-third that of the non-imbibed control. Seed collection also influenced percentage of filled seeds in the 2 fractions. Moly-2 and Moly-3 seed collections had higher percentages of filled seeds in the sinking fraction than the RRC-3 and Moly-1 collections (Figure 6). Overall, the sinking fraction had a higher percentage of filled seeds (12%) than the floating fraction (2%). Neither preparation protocol nor seed collection affected the percent recovery. Percent recovery for the various protocols ranged from 64% to 91%. Mean percent recovery for the 4 seed collections ranged from 70% to 89%.

#### **Germination Study**

Stratification, separation treatment, seed collection, and all interactions of these factors affected water birch germination. On average, seeds in the sinking fraction had the highest total germination (33%) compared to the floating fraction (1%) and non-separated seeds (7%). Separation improved germination by 2X up to over I OX depending on seed collection (Figure 7). Mean germination varied from 5% for Moly-1 to 13% for RRC-1, 15% for Moly-2, and 19% for Chaffee.



*Figure 7:* Effect of separation treatment on water birch germination by seed collection. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

Increased stratification length improved germination slightly, from 11% for non-stratified seeds up to 16% for seeds stratified 56 d, but only seeds in the sinking fraction responded positively to the 56-d stratification treatment (10% increase in germination). The interaction between stratification and separation treatments was inconsistent across seed collections (Figure 8). Germination of non-separated seeds was unaffected by stratification. Sinking fractions of different collections, however, varied considerably in response to stratification. In the Moly-1 collection, the 21-day stratification had no impact, while 56 d of stratification improved germination by 7X. However, in the Moly-2 and RRC collections, germination peaked at 21 d of stratification. Both 21 and 56 d of

stratification reduced germination in seeds from the sinking fraction of the Chaffee collection. Seeds in floating fractions were unaffected by stratification treatments.

#### **Discussion**

Seed refinement techniques must not only increase the percentage of filled (potentially viable) seeds within seed collections but also must increase the percentage of viable seeds as measured by viability testing or, as was the case in this study, germination. In addition, the benefit of an increased percentage of viable seeds in the sinking fraction must not be overweighed by the loss of viable seeds in the floating fraction. Although the 50% relative humidity used in this study is higher than is standard for the procedure, this level was chosen to ensure procedural repeatability at relative humidities higher than in the Chihuahuan desert of southern New Mexico where this study was conducted. Downie and Wang (1992) found that for 3 coniferous species, drying seeds at 50% relative humidity achieved as great a difference in moisture content between live and dead seeds as did drying at 20% relative humidity. In our study, the efficacy of multiple IDS and gravity separation treatments was first assessed by the percentage of filled seeds. The most promising separation treatments were then evaluated across a range of stratification lengths to ensure that improved fill corresponded to improved germination of both species.

Gravity separation of non-imbibed seeds for both thinleaf alder and water birch was superior to IDS treatments in improving the percentage of filled seeds in the sinking fraction. The 2 alder IDS treatments with the longest drying times, 18 and 24 h, resulted in percentages of filled seeds in sinking fractions similar to those following gravity separation of non-imbibed seeds. It is possible that an intermediate duration of drying (between I and 18 h) would have improved the percentage of filled seeds in the sinking fraction. The shorter drying times (O and I h) may have been insufficient to allow unfilled seeds to lose moisture, while the 18- and 24-h drying times may have allowed filled seeds to lose most of the imbibed water. The IDS protocol used in the second experiment improved thinleaf alder germination, with greater improvement in the better quality seed collections. This response is consistent with the results of the refinement study in which the percentage of filled seeds was increased when separation techniques were employed.

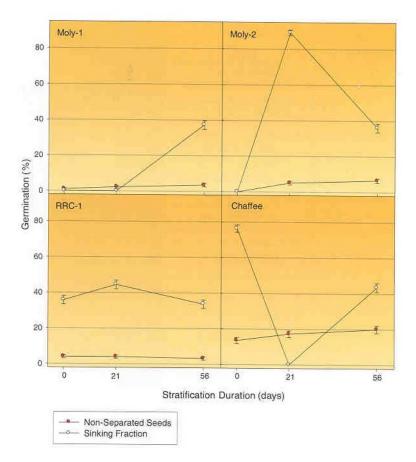


Figure 8: Water birch germination percentage of non-separated and sinking fractions as influenced by stratification length, seed collection, and separation fraction. Error bars represent ± one standard error. Error bars are too small to be visible in some cases.

For water birch, IDS drying times of 0.5 to 2 h may have been too short to allow unfilled seeds to lose sufficient moisture. Insufficient drying results in an increase in the percentage of unfilled seeds in the sinking fraction. The assumption was made that water birch seeds, being small with large integuments, would lose imbibed water at a fast rate. This may not have been the case, as indicated by the higher percentage of empty seeds in the sinking fraction.

The influence of drying time on the efficacy of IDS treatment has been seen in other species. For London plane tree (*Platanus x acerfolia* (Alton) Willd.), drying times from 7.5 h to 24 h improved germination percentage of the sinking fraction (and filled seed percentage) beyond that of the control, but only seeds receiving 24 h of drying as part of an IDS treatment had greater germination than non-treated seeds separated in petroleum ether (Falleri and Pacella 1997).

In our study we observed considerable variability among seed collections in response to separation treatments. This difference was most pronounced between the Reserve and Luna seed collections of thinleaf alder–seed collections with similar baseline percentages of filled seeds. Separation increased the percentage of filled seeds for the Luna collection

to a greater extent than for the Reserve collection. Differences in the rate of moisture loss between the Luna and Reserve collections may have existed during the drying portion of the IDS regimes. Differences among seed collections in response to similar seed refinement techniques (IDS) have been observed in other studies (Donald 1985; Downie and Wang 1992). During seed refinement, some viable filled seeds are lost in the floating fraction (Sweeney and others 1991; Downie and Wang 1992; Falleri and Pacella 1997). The percentage of filled seeds from the original sample recovered in the sinking fraction provides a measure of how efficient the refinement technique is at reducing the number of filled (potentially viable) seeds lost in the floating fraction. For thinleaf alder, high recovery of filled seeds was inversely related to the IDS treatment's ability to remove unfilled seeds. Taylor and Kenny (1985) found a similar trend in an attempt to upgrade germinated cabbage (Brassica oleracea L.) seeds using density gradients. As percent recovery increased, germination percentage in the sinking fraction decreased because of the increased recovery of nongerminable seeds. In the case of water birch, separation technique did not impact percent recovery but did impact the percentage of filled seeds in the sinking fraction.

Effects of stratification on germination of both thinleaf alder and water birch were most pronounced on the sinking fractions of separated seeds, as would be expected, because those fractions contain the highest percentages of filled, viable seeds. In this fraction, the effect of stratification varied among collections of both species. Stratification appears to be advantageous for many species of alder, but the response to stratification can be highly source specific (Schrader and Graves 2000). In paper birch (*Betula papyrifera* Marsh.), New Hampshire and Alaska seed sources have been shown to have different optimum durations of stratification (Bevington and Hoyle 1981). Provenance variation in seed properties and germination is not uncommon and has been reported for a wide range of woody species (Young and Young 1992; Baskin and Baskin 1998).

Depending on a grower's constraints, either greenhouse space or seed supply, the evaluation of seed refinement techniques could be based on 1 of 3 criteria: percentage of filled seeds in the sinking fraction, percentage of filled seeds recovered, or the product generated by multiplying these 2 values. In cases where seed supply is a greater constraint, selection of seed refinement technique may be based solely on the percentage of filled seeds recovered. This seed refinement technique may be less efficient in removing unfilled seeds, but loss of filled seeds would be minimized. In the case where growing space is the greater constraint, the percentage of filled seeds in the sinking fraction would determine the selection of seed refinement technique. When both greenhouse space and seed supply are limited, the product of multiplying percentage of filled seeds in the sinking fraction by the percentage of filled seeds recovered may be used to determine the appropriate protocol to use. The use of this information in conjunction with spreadsheet-based seed sowing programs allows nursery managers to select the best seed refinement technique for their nursery (Wenny 1993; Harrington and Glass 1997).

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## Blunt Panic (Panicum obtusum)

By: LLPMC Staff<sup>10</sup>

Study Number: NMPMC-P-9901-RA

Blunt Panic (commonly called vine mesquite in New Mexico) is a native, stoloniferous, perennial, warm-season grass. It is typically found in sandy or gravelly soil, and chiefly in moist sites along stream and ditch banks. It is fair-to-good forage for livestock and wildlife, and it can withstand heavy grazing. Because of its stoloniferous habit, Blunt panic often grows in dense stands and may be used to stabilize washes and prevent soil erosion. It is in high demand in New Mexico for use in riparian restoration grass seedings. However, it is not currently in commercial production.

Blunt panic seed typically has a low germination rate, mainly due to a low percentage of seed fill. Populations of Blunt panic typically have three ploidy levels; diploid (2n=36), triploid (2n=27) and tetraploid (2n=36 and 2n=40). Of the three ploidy levels present, only the diploid plants were sexual in their mode of reproduction. The triploid and tetraploid plants are facultative apomictics with both sexual and apomictic florets. Blunt panic bulk seed collections were made from 80 collections throughout New Mexico. In 1983, seedlings were transplanted to the field into non-replicated accession rows. Plots were two rows of 14 plants per row. In 1995, seed was hand harvested for each of the 80 accessions in the preliminary evaluation field. In 1997, germination tests were conducted on the 80 accessions.

The previous plant breeder selected the accessions that displayed the best seed fill and germination rates (40–60 percent). These accessions are listed in the following table:

Accession	Origin (NM County)
9027854	Socorro
9029111	Sandoval
399274	?
9003860	?
9023213	Valencia
9027836	Colfax
9027930	Rio Arriba
9023296	Union
9029719	Union
9003848	?

<sup>&</sup>lt;sup>10</sup> David Dreesen, Gregory Fenchel, Danny Goodson

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Seed from the selected accessions was taken to the Tucson Plant Materials Center where the plant breeder will continue the development of a commercial release with an improved seed germination rate.

The original ¼-acre planting of the 80 accessions is still alive and growing vigorously. The ten selections that were made were not randomly located throughout the planting as one would expect. All but two accessions were located only on the outside rows of the planting. These outside rows generally receive slightly more water due to a border affect, and this can significantly improve seed fill and germination rate if plants are moisture stressed. Subsequently, the planting has been heavily irrigated and fertilized in 2004 to encourage maximum seed production. Seed will be bulk harvested and tested for germination rate. If seed fill and germination rate is satisfactory (above 50%), the seed will be used to establish a new seed production field. This is necessary because there is such a high, local demand for this commonly occurring species. This seed will be provided to interested growers as a selected plant material release.

If the seed germination rate is poor after bulking all the lots, we will increase the two accessions through vegetative means that performed well in the interior of the planting. This seed will be planted in a new, isolated planting and will be measured for seed fill and germination rate.

## Giant Sacaton (Sporobolus wrightii)

By: LLPMC Staff<sup>11</sup>

Study Number: NMPMC-P-8401-CP

Giant sacaton is a native, robust, perennial warm-season bunchgrass. It is found throughout the southwestern United States, usually occurring on low alluvial flats and flood plains. It is useful forage for livestock and wildlife. Under irrigation, Giant sacaton may reach heights exceeding 3 m. Mature plants range in height from 1 m to 4 m. Based upon its density and height, and it has the potential as a windbreak plant for irrigated cropland.

Seed collections of Giant sacaton were taken from 37 locations throughout New Mexico. These collections were used to establish non-replicated, accession rows that consisted of 520 plants in a field at the Los Lunas Plant Materials Center. Based on a visual evaluation of vigor and height, ten superior plants were selected. Each selected superior plant came from a separate accession to maintain a diverse population. From these ten plants, one super selection was made. A hybrid, cross-planting was established as an attempt to improve the height of the progeny.

In 1992, colonel shoots of each selected plant were planted into a hybrid, seed-production block with the super plant as the male pollinator. In 1995, seed was hand-harvested from each female parent. In 1996, this seed was used to establish an evaluation planting that contained both parents and progeny. The progeny were derived from seed, and the parents were vegetatively propagated. Both sets of plants were grown in 6-inch square pots for eight months in an attempt to equalize carbohydrate reserves in the seed derived plants and the clones. The planting design was an 8-replicated, split-plot, randomized block design. Each replication consists of 20 plants spaced on 10-ft. centers. A plot consists of a parent and the progeny plant.

The planting is mowed in the winter to remove plant liter from the previous year. By the end of the third growing season, the leaf blades of most plants had approached 3 m in height. When the plants are flowering they may approach 4 m.

In August 2002, the planting was evaluated for leaf height, basal width, and appearance. A separate, paired, T-test statistical analysis was performed on each replication comparing the height of each parent to its progeny. The progeny and parent plants were not significantly different in height (alpha .05). However, there appears to be a difference in leaf blade width, color, and uprightness between the parents and progeny plants. The cloned parent plants remain identical to their source where the progeny plants seem to have random variation. The planting will be evaluated again in 2005.

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<sup>&</sup>lt;sup>11</sup> David Dreesen, Gregory Fenchel, Danny Goodson

NRCS Field Offices continue to be very interested in using this plant material for wind strip plantings. In 2004, transplants were provided to the Field Offices in Clayton, Estancia, and Grants, NM, and on the Navajo Reservation in McKinley County and Isleta Reservation in Valencia County, New Mexico. This distribution continues to promote the utility of this plant material for use in wind strips, and additionally defines its range of adaptability.

Field maintenance for the parent and parent and progeny test plantings consisted of the following.

#### 2003 Treatment and Harvest

Weed control was performed throughout the growing season to keep the fields clean and promote vigorous growth of the plantings.

Action	Date	
Irrigation (3" applications)	4/30, 5/19, 6/6, 6/25, 7/16, 8/19, and 10/16	
Pre-emergent herbicide *	5/16/2003	
Pesticide application, 2,4-D	3/7/2003	
Fertilizer		
30 pounds of Nitrogen	5/15/2003	
40 pounds of Nitrogen	6/24/2003	
40 pounds of Phosphorous	5/15/2003	
40 pounds of Phosphorous	6/24/2003	
Harvest completed (Field 10 only)*	10/21/2003	
Swathed	1/13, 11/25	
Baled	1/15	

<sup>\*</sup> No other fields were harvested in 2003.

# New Mexico Needlegrass (*Stipa neomexicana*) and Needleandthread (*Hesperostipa comata*)

By: LLPMC Staff<sup>12</sup>

Study Number: NMPMC-P-9504-CR

New Mexico needlegrass and needleandthread are native, perennial, cool-season grasses that are tolerant to only 8–10 inches of annual precipitation. They provide fair-to-good forage for livestock and wildlife. However, their flower has long awns, and it may prove injurious to livestock. Both are commonly found on calcareous and sandy soils. The breeding system of needleandthread is self-pollination. New Mexico needlegrass also appears to be primarily self-pollinating.

Seed from six New Mexico needlegrass and 61 needleandthread accessions were obtained from bulk seed collections throughout New Mexico, Arizona, and Montana. In 1985, these bulk collections were established in a field at the LLPMC and into non-replicated accession plots. The plots consisted of two rows of 14 plants each. Fifteen needleandthread accessions and three New Mexico needlegrass accessions were selected based on survival, foliage height, basal width and visual vigor. Seed was bulk harvested from all plants of the selected accessions.

In 1994, a replicated entry evaluation of the selected accessions was established at two sites at the LLPMC. The experimental units consisted of a plot containing two plants. The experiment at both sites was conducted in a randomized complete block with nine replications. Site #1 had salinity levels ranging from 3.3 to 4.5 mmhos, cm<sup>-1</sup>, and Site #2 ranged from 0.42 to 0.44 mmhos, cm<sup>-1</sup>. In 1996, Site #1 was abandoned because the site was weedy, and a majority of the plants had died possibly due to the high soil salinity. Site #2 did well and contains healthy plants.

In 2005, superior plants will be selected from the 18 remaining accessions. Plants from both species will be selected, vegetatively increased, and planted into two separate seed increase fields for continued testing. This testing will include off-center plantings.

#### 2003 Treatment and Harvest

Weed control was performed throughout growing season to keep the field clean and to promote vigorous growth of the planting. No evaluations were made in 2003 or 2004.

Action	Date
Irrigations 3" water application	3/13, 4/7, 5/23, 6/4, 6/26, 7/17, 8/21,
	10/22/2003
Fertilizer	2/11/2003

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70 lbs Nitrogen 120 lbs Phosphorous	
Mow	3/26, 7/11, 11/12/2003

### Prairie Junegrass (Koelaria marcantha)

By:LLPMC Staff<sup>13</sup>

Study Number: NMPMC-P-9801-RA

Prairie junegrass is a cool-season, perennial grass native to North America and temperate areas of Europe. Its range extends across the western, central and northeastern United States. In New Mexico, it occurs at elevations between 6,000 and 11,000 feet. It provides excellent forage for all classes of livestock and wildlife. Populations of Prairie junegrass may be either diploid (2n=14) or tetraploid (2n=28). Researchers have reported that ploidy level within a population will increase with drought stress, and that tetraploid populations may reach anthesis as much as 21 days before their diploid counterpart. Collections of Prairie junegrass were made from 98 locations throughout New Mexico. The populations from New Mexico and two exotic populations were planted into non-replicated, preliminary evaluation in 1984. These plots consisted of two rows of 14 plants. In 1989, three early-flowering and three late-flowering accessions were visually selected from this evaluation. The ploidy level of the selected accessions is unknown. The three early-maturing accessions were collected from similar areas, suggesting they may have the same ploidy (Table 1). Two of the late-maturing accessions are from Torrance County, NM suggesting that they may have the same ploidy level.

Table 1: Collection site information for Prairie junegrass (*Koelaria macrantha*) accessions selected in 1989 for vigor and forage value.

111 1707 101 1150	or and forage var	401		
Accession or PI	Maturity	Origin	MLRA	Elevation
Number				
9035465	Early	Catron	39	6519
9035466	Early	Catron	39	7483
9035467	Early	Catron	39	6598
9035559	Late	Torrance	70	6798
9035594	Late	Torrance	70	6699
PI-207489	Late	Afghanistan	-	-

In 1989, polycross blocks were established for the early-maturing and late-maturing accessions. Plants for both types of polycross blocks were derived from the original collections.

In 1997, the polycross block for the late-maturing accessions did not perform as expected, and was abandoned. In 1998, superior plants were selected from the early-maturing polycross block established in 1989. Seed was collected from these plants, and clones were established from the parents.

In 1999, an evaluation planting was established to compare the parents to the progeny. This planting is replicated six times and is a latin square design. These accessions were

<sup>&</sup>lt;sup>13</sup> David Dreesen, Gregory Fenchel, Danny Goodson

visually evaluated for forage and seed yield in 2003 and 2004. The progeny appear to be breeding true to the parents for there was no visual difference. Subsequently the planting will be used for seed production to support further field testing that will compare the performance of this material to other available commercial sources. The range of adaptability of this germplasm will be determined during field testing, and if it displays superior performance, the germplasm will be released to the commercial seed industry.

#### 2003 Treatment and Harvest

Field maintenance included the following:

- Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.
- This planting was not evaluated in 2003. Evaluations will be completed in 2004 to work towards a possible release of this species.

Action	Date
Irrigation 3" application	2/12, 4/8, 4/28, 5/16, 6/2, 6/23, 7/11, 7/29,
	8/1, 9/29, 10/30/2003
Pre-emergent herbicide	4/4/2003
Fertilizer	2003
80 lbs Nitrogen	
40 lbs Phosphorous	

### **Field Plantings**

By: Danny Goodson 14

### Evaluate Forage Triticale Planting at Jeff Glenn Farm-Study Number: NMPMC-T-0001-PA

In prior discussions with Mr. Glenn and the staff at the Silver City USDA-NRCS Field Office, a need was seen for trials of species of pasture grasses other than the triticale Mr. Glenn has been traditionally using. A forage triticale trial was not established on the Glenn Farm in Silver City, NM in 2003.

In 2003, the Los Lunas Plant Materials Center was able to acquire San Marcos gamagrass seed from the Knox City Plant Materials Center. This seed was sent to Mr. Glenn in the late winter of 2003 for direct seeding into one of his pastures. Mr. Glenn seeded one acre of his three-acre pasture using the San Marcos gamagrass seed. On his own he purchased enough gamagrass seed to seed the remaining two acres.

On September 24, 2003, a visual inspection of the gamagrass seeding was completed. The acre of San Marcos gamagrass had a 35% stand and was noticeably more vigorous in areas that appeared to be receiving more moisture. The remaining two acres of gamagrass also had a stand of 35%, but it appeared to have a little more growth than the San Marcos gamagrass. Both plantings seemed to have a better germination rate where moisture levels appeared to be higher (such as in depressions). Growth of most plants averaged 8 to 10 inches, and two plants had established seed stalks. The planting will be re-evaluated in 2004.

# Tatum Windstrip Plantings-Tatum Memorial Cemetery Windstrip Planting Study Number: NMPMC-F-0201-WI

On September 26, 2003 the Giant sacaton windstrip planted at the cemetery was evaluated for survival and growth rates. The planting appears to be in fair condition. Plants averaged 42 inches in height and 25 inches in width. The survival rate has remained the same since evaluating it in 2002. The limited amount of growth could be caused by inconsistent irrigation throughout the entire planting and by competition with the existing lawn.

This planting will be re-evaluated in 2004.

### **Tatum Town Park Windstrip Planting**

On June 12, 2003 a Giant sacaton windstrip planting was installed at the town park in Tatum, New Mexico. The windstrip planting is a joint effort between the town of Tatum and Lea Soil and Water Conservation District with assistance from the Sureste Resource

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Conservation and Development Office. The purpose windstrip is to protect the park area against wind erosion, to provide landscaping aesthetics, and to test the adaptability of Giant sacaton in this area of New Mexico.

The planting consists of 300 Giant sacaton transplants that were planted along the north, east and west sides of the town park. The planting receives its irrigation from a sprinkler system that was already located in the park.

On September 26, 2003 the planting was evaluated for survival and growth rates. The planting seems to be struggling with the location of the planting. Competition of the existing lawn appears to be slowing the growth of the plants. The plants may also have been mowed accidentally during maintenance of the lawn area. The plants have averaged only 10 inches in height, but they have a survival rate if 100 percent.

This planting will be re-evaluated in 2004.

# Field Maintenance at the Los Lunas Plant Materials Center

By: Danny Goodson<sup>15</sup>

### Alkali Muhly-Study Number: NMPMC-P-8301-RA

This Alkali muhly collection is being evaluated for its potential as a variety release. Evaluation of this collection of Alkali muhly will be completed in 2004.

#### 2003 Treatment and Harvest

Action	Date
Irrigation (3" applications)	5/23, 6/4, 6/26, 7/17, 8/21 and 10/22/2003
Field burned	4/4/2003
Pre-emergent herbicide *	5/19/2003
Fertilizer	2003
100 pounds of Nitrogen	
120 pounds of Phosphorous	
Harvest completed	2003

<sup>\*</sup> Note that this species is not tolerant to 2,4-D types of herbicides. Severe die-back of the foliage has been observed after applying this herbicide.

### **Evaluation of National Arboretum American Elm Selections-Study Number: NMPMC-F-0201-OT**

Two varieties of American elm were obtained from the National Arboretum in the spring of 2002. The National Arboretum shipped three rooted plants of two varieties, Valley Forge and New Harmony to the LLPMC for evaluation in our hardiness zone. On August 15, 2002 the six trees were transplanted into Field 26S on the LLPMC.

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

The planting will be evaluated for growth and survival in 2004.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	4/8, 4/18, 4/25, 5/16, 5/23, 6/4, 6/19, 6/27, 7/11, 7/17, 8/1, 8/19, 10/30/2003
Pre-emergent herbicide	5/16/2003

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Action	Date	
Fertilizer	2003	
40 lbs Nitrogen		
40 lbs Phosphorous		

### Propagation of Autumn Amber-Study Number: NMPMC-P-9803-UR

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

Evaluation of propagation techniques will be performed in 2004 as required.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	4/8, 5/5, 5/19, 6/23, 7/16, 8/19, 10/17/2003
Pre-emergent herbicide	4/4/2003

### Hope Desert Willow Stock Plant Production-Study Number: NMPMC-P-0102-UR

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

Seed was not harvested in 2003 from this planting.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	4/8, 5/16, 6/2, 6/25, 7/11, 7/29, 8/19,
	10/15/2003
Pre-emergent herbicide	5/16/2003
Fertilizer	2003
40 lbs Nitrogen	
40 lbs Phosphorous	

### Regal Desert Willow Stock Plant Production-Study Number: NMPMC-P-0101-UR

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

Seed was not harvested in 2003 from this planting.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	4/8, 5/16, 6/2, 6/25, 7/11, 7/29, 8/19,
	10/15/2003
Pre-emergent herbicide	5/16/2003
Fertilizer	2003
40 lbs Nitrogen	
40 lbs Phosphorous	

### Initial Evaluation of Species from Four Corners Region-Study Number: NMPMC-P-9505-CR

Plantings of different species collected in the Four Corners region of New Mexico were not evaluated in 2003.

Weed control was performed throughout the growing season to keep the fields clean and promote vigorous growth of the plantings.

Evaluations will be completed in 2004 to work towards a possible release of selected species.

#### 2003 Treatment and Harvest

Action	Date
Shrub collections	
Irrigations 3" water application	4/18, 6/4, 6/26, 8/19, 10/12/2003
Desert needlegrass and Slender wheatgrass	
Irrigations 3" water application	2/21, 4/17, 6/4, 6/26, 8/19, 10/22/2003
Fertilizer, 50 lbs Phosphorous	2/11/2003
Harvested Slender wheatgrass	6/4/2003
Mow	11/12/2003
Mexican whitesage, Buckwheat and White prairieclover	
Irrigation 3" water application	6/4, 6/26, 8/19, 10/22/2003
Mow	11/12/2003
Muttongrass	
Irrigation 3" water application	2/19, 4/8, 4/30, 6/4, 6/26, 8/19, 10/12/2003
Fertilizer Applied, 50 lbs Phosphorous	2/11/2003

### Little Bluestem Initial Evaluation Planting-Study Number: NMPMC-P-9101-RA

In 2003, this planting was not evaluated and seed was not harvested.

Evaluations will be completed in 2004 to work towards a possible release of this species.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	5/22, 6/11, 7/1, 7/18, 8/15, 10/17/2003
Swathed	11/25/2003
Baled	11/26/2003

### Evaluation of Mexican Whitesage-Study Number: NMPMC-9801-WL

This Mexican Whitesage collection is being evaluated for its potential as a variety release.

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

The plants produced seed in 2003, but no harvest was attempted. Seed harvest will be completed in 2004.

#### 2003 Treatment and Harvest

Action	Date
Irrigation (3" application)	5/16, 6/23, 8/19, 10/16/2003
Pre-emergent Herbicide	5/16/2003
Plants swathed	1/13/2003
Plants baled	1/15/2003

### Sandhill Muhly-Study Number: NMPMC-P-9601

This collection of Sandhill mully is being evaluated for its potential as a variety release. Evaluation of this collection of Sandhill mully will be completed in 2004.

#### 2003 Treatment and Harvest

Action	Date
Irrigation (3" applications)	5/19, 6/6, 6/24, 7/16, 8/19 and 10/16/2003
Pre-emergent herbicide	5/16/2003
Fertilizer	2003
120 lbs Nitrogen	
80 lbs Phosphorous	
Harvest completed	2003

### **Evaluation of Single Leaf Ash and Fragrant Ash-Study Number: NMPMC-P-9804-UR**

Weed control was performed throughout the growing season to keep the field clean and promote vigorous growth of the planting.

Evaluation of the accessions will continue in 2004 for possible release.

### 2003 Treatment and Harvest

Action	Date
Irrigation 3" application	4/8, 5/16, 6/2, 6/25, 7/11, 7/29, 8/19,
	10/15/2003
Pre-emergent herbicide	5/16/2003
Fertilizer	2003
40 lbs Nitrogen	
40 lbs Phosphorous	

## Developing a Plan for Evaluating IE Tobosa Planting-Study Number: NMPMC-P-8301-RA

The culms planted in 2003 did not survive the transplanting process. The plants will have culms taken in 2004, and they will be placed in containers in the greenhouse. The plants grown from these culms will be planted into an increase production block at the Los Lunas Plant Materials Center (LLPMC).

Evaluation will continue on these superior selections, and work will continue towards a release of Tobosa grass.

### **2003 Annual Seed Production**

Project Number/Name	Field #	Acres	Planting Date	Fertilizer Applications	2003 Irrigation Dates (3" Application)	Harvest Date	Harvest (Cleaned Wt.)
NMPMC-S-8801-RA Alma Blue grama Foundation Quality Seed	7 and 8	2.7	1983 and 1988	80 lbs Nitrogen 80 lbs Phosphorous	5/22, 6/17, 7/2, 7/25, 8/21, 10/2/2003	10/23/2003	N/A
NMPMC-S-9701-RA Nogal Black grama Foundation Seed Field	21N	1.3	1997	160 lbs Nitrogen 120 lbs Phosphorous	5/17, 6/5, 6/26, 7/14, 8/6, 8/25, 10/2/2003	11/3/2003	122 lbs
NMPMC-S-9803-RA San Juan Narrowleaf Penstemon Foundation Seed Field	348	0.85	1998	70 lbs Nitrogen 30 lbs Phosphorous		7/17/2003	130 lbs
NMPMC-S-8803-RA Elida Sand bluestem Breeder Quality Seed	31S	0.14	1988	30 lbs Nitrogen 40 lbs Phosphorous	5/20, 6/11, 7/3, 7/23, 8/27, 10/17/2003		N/A
NMPMC-S-RA Grant Cane bluestem Foundation Quality Seed	9	1.75	1999 and 2000	110 lbs Nitrogen 80 lbs Phosphorous	4/11, 5/9, 6/5, 6/27, 7/18, 8/8, 10/1/2003	7/17- 8/26/2003	N/A
NMPMC-S-7801-RA Hachita Blue grama Foundation Quality Seed	16	2.0	1963	150 lbs Nitrogen 120 lbs Phosphorous	5/22, 6/11, 7/1, 7/29, 9/17/2003	10/28/2003	N/A
NMPMC-S-6501-RA Jose Tall wheatgrass Foundation Quality Seed	16	1.0	1965	110 lbs Nitrogen 120 lbs	2/12, 4/17, 5/19, 6/6, 7/1, 7/29, 9/19/2003	8/28/2003	90 lbs

### **2003 Annual Seed Production**

Project Number/Name	Field #	Acres	Planting Date	Fertilizer Applications	2003 Irrigation Dates (3" Application)	Harvest Date	Harvest (Cleaned Wt.)
				Phosphorous			
NMPMC-S-9902-RA Largo Tall wheatgrass Foundation Quality Seed	12	0.45	5/3/1999	120 lbs Nitrogen 120 lbs Phosphorous	2/2, 4/8, 4/28, 5/19, 6/4, 7/1, 7/17, 8/21, 9/29, 11/7/2003	9/19/2003	N/A
NMPMC-S-9401-RA Paloma Indian ricegrass Foundation Quality Seed	8 and 25N	0.25 and 0.89	1994 and 2000	120 lbs Nitrogen 120 lbs Phosphorous	1/7, 2/12, 4/7, 4/21, 5/13, 6/6, 6/27, 7/18, 8/15, 10/2, 11/7/2003	6/2-5/2003	181 lbs
NMPMC-S-8802-RA Pastura Little bluestem Breeder Quality Seed	31S	0.14	1988	30 lbs Nitrogen 40 lbs Phosphorous	5/21, 6/11, 7/3, 7/23, 8/27, 10/16/2003		N/A
NMPMC-S-6701-RA Salado Alkali sacaton Foundation Quality Seed	15	1.0	1975	90 lbs Nitrogen 40 lbs Phosphorous	5/13, 6/5, 6/25, 8/1/2003	7/29/2003	N/A
NMPMC-S-9903-RA Tusas Bottlebrush squirreltail Foundation Quality Seed	13	.95	5/3/1999	180 lbs Nitrogen 260 lbs Phosphorous	1/17, 3/13, 4/3, 4/21,5/13, 6/26, 7/18, 8/15, 10/3, 11/7/2003	6/12/2003	126 lbs
NMPMC-S-9401-RA Vaughn Sideoats grama Foundation Quality Seed	25N	.33	5/26/1992	100 lbs Nitrogen 40 lbs Phosphorous	4/15, 5/13, 6/4, 6/24, 7/3, 7/14, 8/1, 10/15/2003	9/23/2003	N/A
NMPMC-S-7501-RA Viva Galleta grass	13	3.20	1975	80 lbs Nitrogen	5/20, 6/5, 6/27, 7/17, 8/21/2003	7/11/2003	N/A

### **2003 Annual Seed Production**

Project Number/Name	Field #	Acres	Planting Date	Fertilizer Applications	2003 Irrigation Dates (3" Application)	Harvest Date	Harvest (Cleaned Wt.)
Foundation Quality Seed				40 lbs Phosphorous			
NMPMC-S-9902-RI Seed Increase of Alkali Muhly	12	0.25	1999	120 lbs Nitrogen 120 lbs Phosphorus	5/23, 6/4, 6/26, 7/17, 8/21, 10/22/2003	10/21/2003	N/A
NMPMC-S-0403-WO Produce seed of Grand Canyon National Park Muttongrass	25S	0.90	2003	40 lbs Nitrogen 40 lbs Phosphorous	10/20, 10/30, 11/10, 12/5/2003		No harvest
NMPMC-S-0307-RA Produce Salado Alkali Sacaton Foundation seed	11 and 19	1.0 and 0.72	2003	30 lbs Nitrogen 40 lbs Phosphorous	7/22, 7/23, 7/25, 8/1, 8/8, 8/15, 9/3, 9/19, 10/1, 10/30, 12/31/2003		No harvest
NMPMC-S-0301-RA Viva Galleta Foundation Seed field	19	2.4	2003		9/3, 9/16, 9/18, 9/23, 9/29, 9/30, 10/24, 12/5/2003		No harvest
NMPMC-S-0004-WO Produce foundation quality seed of Grand Canyon muttongrass	20N	1.0	2000 and 2002	350 lbs Nitrogen 250 lbs Phosphorous	1/7, 2/12, 3/12, 4/3, 4/15, 4/25, 5/7, 5/20, 6/4, 6/25, 7/15, 8/1, 9/29, 10/15, 12/5/2003	5/16/2003	33 lbs
NMPMC-S-0003-RA Produce foundation quality seed of Grand Canyon blue grama	20N	0.50	2000	170 lbs Nitrogen 130 lbs Phosphorous	4/16, 5/20, 6/4, 6/26, 7/15, 8/11, 10/16/2003	10/27/2003	20 lbs

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